

Determination of Fixed Charge Density Inside Articular Cartilage from Unconfined Compression and Validation with Chemical Assay

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INTRODUCTION

The early stage of the osteoarthritis (OA) is associated with the fibrillation of collagen and the loss of proteoglycans (PGs). Biomechanically, PGs are primarily responsible for the compressive properties because the fixed charge density (FCD) on PGs generates the Donnan osmotic pressure inside the tissue [1]. The collagen fibrils have much higher modulus at tension than that under compression; this is conferred to the tissue and known as tension-compression nonlinearity (TCN). The osmotic effect of PGs inside a charged mixture have been modeled with the triphasic theory [2], while the TCN has been used to explain the high initial force response under unconfined compression [3] and often modeled with the conewise linear elasticity (CLE) model [4]. A constitutive model accounting for the contributions from the two major biochemical components of the tissue will provide important insights into cartilage mechanical functions. Therefore, the objectives of this study will be: 1) to develop and solve the CLE incorporated triphasic equations *analytically*, and 2) to validate this triphasic-CLE model by determining the FCD from unconfined stress-relaxation experimental data with efficient analytic solutions and comparing the results with those from chemical assay.

METHODS

Experiments: Ten cylindrical disks (diameter ~3mm) were punched out from femoral grooves of three healthy, 18-month-old skeletally matured adult bovine knee joints. The cartilage sample was lubricated with synovial fluid and immersed in 0.15M phosphate buffered saline (PBS) with protease inhibitor (PI) cocktail. The sample was compressed between two impermeable flat ceramic platens with an Instron Microtester. After a tare load of 2.5 grams for 5 minutes, the sample was ramped to 10% tissue strain in a step fashion (~ 0.4s), and then the displacement maintained constant for about 30 minutes. The sample was allowed for a full recover and equilibrate in 2M PBS + PI solution for 2 hours, followed by the same unconfined test procedure in 2M PBS + PI solution [5]. Finally, the samples were subjected to the DMMB assay to determine the GAG content for calculating FCD.

Theoretical Analysis: The platens are assumed to be frictionless and radial expansion is unconstrained. The sample is assumed to be homogenous, and the elastic solid matrix experiences infinitesimal deformation. We further hypothesized that the preferred material directions of these samples are along circumferential, radial and axial directions. Please note that these simplifying assumptions are conceptually similar to several previous studies [3,6]. The CLE model was incorporated into the triphasic theory [2] to describe the TCN of the solid matrix of articular cartilage. Following a regular perturbation procedure, we have been able to reduce the

number of the partial differential equations into just two and solved them analytically with the Laplace transform method [7]. A least-squares curve-fitting algorithm was used to find the best-fit material parameters by matching the

experimental data and the theoretical prediction. We curve-fitted the force responses of the tissue at 0.15M and at 2M simultaneously while using different values for the permeability (*i.e.*, k_{2M} and $k_{0.15M}$) at these two different solutions [5]. The intrinsic Poisson's ratio of the solid matrix is assumed to be 0.05. We curve-fitted for five intrinsic parameters: tensile and compressive aggregate moduli H_{+a} and H_{-a} , k_{2M} , $k_{0.15M}$, and FCD.

RESULTS AND CONCLUSION

A typical set of stress relaxation data and their curve-fits at 0.15M and 2M are shown in **Fig. 1**. Good agreements have been found between experimental data and theoretical prediction. The average values and their standard deviations for the curve-fitted parameters are listed in **Table 1**. All these values are in the normal range.

Table 1. Mechanochemical properties of the tissue samples.

H_{+a} (MPa)	H_{-a} (MPa)	K_{2M} ($10^{-15} \text{ m}^2/(\text{N}\cdot\text{s})$)	$K_{0.15M}$ ($10^{-15} \text{ m}^2/(\text{N}\cdot\text{s})$)	FCD (mEq/ml)
1.8 ± 1.3	0.21 ± 0.14	3.1 ± 1.8	4.5 ± 3.4	0.20 ± 0.13

The FCD predicted from triphasic-CLE model is 0.20 ± 0.13 mEq/ml, while the chemical assay gives an average value of 0.16 ± 0.08 mEq/ml. A significant correlation has been found between these two values ($R^2=0.72$, $p=0.002$; **Fig. 2**) with a slope close to 1 (1.3 ± 0.3) and an intercept close to zero (-0.02 ± 0.05 mEq/ml).

In conclusion, with triphasic-CLE model, we have

been able to determine the tensile aggregate modulus and the FCD simultaneously and evaluate how collagen and proteoglycans contribute to the mechanical properties of cartilage. The results may provide extra insights into the etiology of OA, and help the diagnosis of early stage OA.

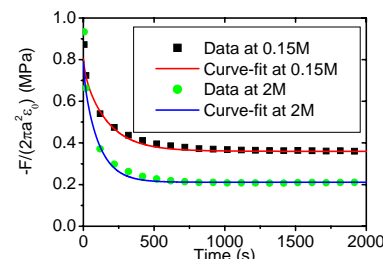


Figure 1. A set of typical unconfined compression stress relaxation histories of articular cartilage bathed in 0.15M and 2M saline solution.

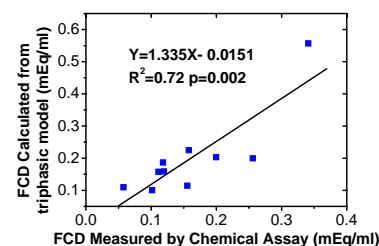


Figure 2. Correlation of FCD calculated from the triphasic-CLE model of the unconfined compression of articular cartilage and the FCD determined by the chemical (DMMB) assay.

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